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Survival of Enteric Viruses Under Natural Conditions in a Subarctic River

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16. ABSTRACT

The survival of enteric viruses was studied in the vicinity of Fairbanks, Alaska at selected stations along a 317-km section of the Tanana River. This section was located downstream from all known domestic wastewater sources and was effectively sealed by a total ice cover. The mean flow time through the region was 7.1 days, during which initial viral population showed a relative survival rate of 34%. The tracing of native viruses at such great distances in the complete absence of other point and nonpoint viral sources has not been previously reported. Of the two methods of virus concentration used, viral recoveries from the disk adsorption virus elution procedure were far greater than those achieved with the Aquella system employed at that time. The fact that the ratio of enteric viruses to fecal indicator bacteria was not constant clearly inferred that these bacteria were not an effectual measure of virus concentration. The persistence of fecal coliforms and fecal streptococci, however, attested to the microbiological health risk involved.

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Survival of Enteric Viruses Under Natural Conditions in a Subarctic River

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The survival of enteric viruses was studied in the vicinity of Fairbanks, Alaska at selected stations along a 317-km section of the Tanana River. This section was located downstream from all known domestic wastewater sources and was effectively sealed by a total ice cover. The mean flow time through the region was 7.1 days, during which initial viral population showed a relative survival rate of 34%. The tracing of native viruses at such great distances in the complete absence of other point and nonpoint viral sources has not been previously reported. Of the two methods of virus concentration used, viral recoveries from the disk adsorption virus elution procedure were far greater than those achieved with the Aquella system employed at that time. The fact that the ratio of enteric viruses to fecal indicator bacteria was not constant clearly inferred that these bacteria were not an effectual measure of virus concentration. The persistence of fecal coliforms and fecal streptococci, however, attested to the microbiological health risk involved.

Exposure of humans to viral infection, including the risks associated with the persistence of human enteric viruses in natural waters, is always a public health concern. Enteric viruses are well adapted to prolonged survival outside susceptible hosts (4), which led Shuval (16) to suggest that their durability be compared to that of refractory chemicals found in wastewater effluents. These viruses, however, are subject to much greater environmental control than are viruses spread by the respiratory route. As pointed out by Sproul (18), their control may be achieved at any level by present-day technology. Where high costs are involved, as with treatment technology, the question of what constitutes reasonable protection becomes an issue. The extent to which enteric viruses are transported from their point of discharge must be weighed against the cost-benefit values of differing levels of treatment. Thus, for the present, the clouded issue of waterborne viral infections generates superficial and often conflicting judgments which must serve as a basis for determining safe public health practices.

There are formidable obstacles in identifying the point source of viruses that are detected at any distance downstream from their discharge. Consequently, monitoring the capacity of the enteric virus to survive in natural waters has been restricted essentially to laboratory evaluations. The few attempts to follow the course of these viruses in natural waters have noted their presence at points of up to 25 km from the main source of pollution (17). In contrast to these earlier investigations, this study was conducted on a 317-km reach downstream from all sources of domestic pollution. Moreover, ice totally covered the river, effectively sealing it against possible extraneous viral contamination, thus affording the opportunity to trace indigenous viruses at considerable distances from their source.

MATERIALS AND METHODS

Sampling sites. A collaborative study on a section of the Tanana River during the winter of 1975 has been described by Davenport et al. (7). Figure 1 shows those stations that were sampled between February 24 (day 1) and March 7 (day 12) for the presence of viruses. These sampling stations ranged from T-100, located near the confluence of the Tanana and Yukon Rivers, to station T-900, located about 32 km east of Fairbanks, Alaska. The C-100 station, located on the Chena River 7 km from its confluence with the Tanana River, was also sampled. Water temperature was 0°C, and all samples were collected through holes cut in the ice as previously reported (7).

Samples were also taken of discharges from all sewage treatment plants that indirectly emptied into this section of the river. These included Fairbanks, College Utilities, and Fort Wainwright plants, which emptied into the Chena River, and Eielson Air Force Base and North Pole plants, which emptied into Moose Creek, which in turn flowed into the Tanana River just below station T-900. Downstream from the junction of the Chena and Tanana Rivers to the

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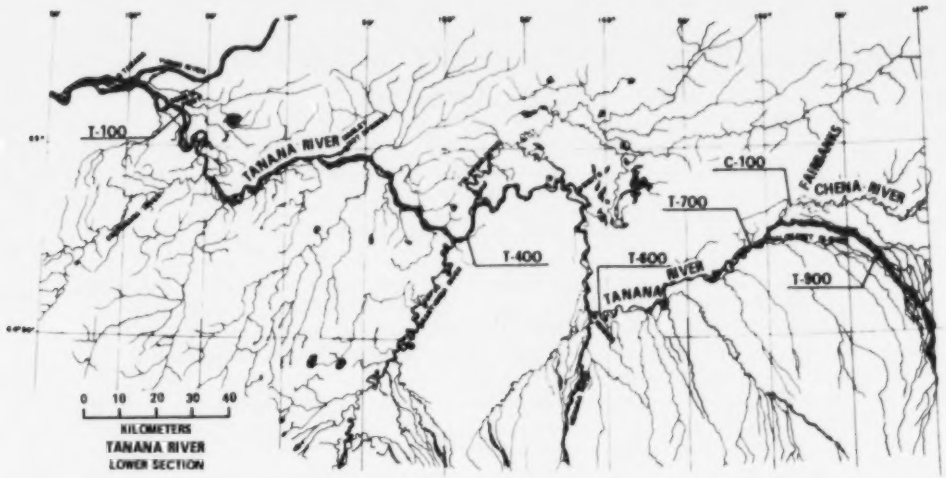

FIG. 1. *Map of the lower Tanana River showing the location of sample stations.*

furthest station studied (T-100), a distance of 330 km, the waterway received no new domestic sewage effluents. To permit a thorough, natural blending of these waters, sampling was initiated 13 km below this junction at station T-700. This site yielded the baseline data from which relative survival rates from successive sampling stations (T-600, T-400, and T-100) were determined.

Cell cultures. A continuous African green (*Cercopithecus aethiops*) monkey kidney cell line, designated as BGM (1, 6), was used at passages 109 to 120 for all virus isolations. The methods of propagation have been described previously (6), except that cells were grown in an equal mixture of minimum essential medium (Eagle) with Hanks salts, L-glutamine, and nonessential amino acids (Grand Island Biological Co.) and L-15 medium (Leibovitz) with L-glutamine (KC Biological) supplemented with 10% calf serum and 7.5% $NaHCO_3$.

Virus assay. All assays were performed in screw-capped bottles by the plaque method (6). A 1.0-ml sample was inoculated onto BGM monolayers. Bottles were examined over a 3-week period for the appearance of plaques. All plaques from the Tanana River samples were picked. Samples from the Chena River and sewage treatment plants yielded far too many plaques to permit picking all of them. In these samples an initial pick of up to 10 plaques was made followed by additional random picks throughout the incubation period. The plaques picked totaled between 22 and 47% of the final count, depending on availability of well-isolated plaques. The isolated plaques were inoculated in tube cultures, and the recovered viruses were propagated and stored for later identification.

Virus identification. Isolated viruses were propagated in cell culture until cytopathogenic effects of 4+ were discerned. Each of the culture isolates was frozen and stored at −70°C until titrated. Those cell culture tubes receiving the highest dilution at which cytopathogenic effects were detected were appropriately diluted, and each was assayed against an antisera pool containing the three poliovirus antisera. The cultures that were not identified by this pool were assayed against the Lim-Benyesh-Melnick pools as described elsewhere (11).

Sample preparation and concentration. Appropriate samples were collected from each station as previously described (7) and processed by the disk adsorption virus elution procedure (Fig. 2) by using the low-pH method described by Rao et al. (13). The filters used were the 293-mm AP15 and AP20 prefilters and the 142-mm, 0.45-μm virus adsorption filter (Millipore Corp.). Filters were autoclaved for 15 min at 121°C. To prevent adsorption of virus to prefilters AP15 and AP20, we treated the prefilters with 0.1% Tween 80 and washed them with distilled water. The prefilters were stacked in a holder with AP20 positioned above the AP15. Sewage samples were processed at pH 3.5 as previously described (14). Water samples were similarly processed, except that pH was adjusted and $MgCl_2$ was added before prefiltration. When clogged, the prefilters were replaced, and clogged prefilters were stored for further processing.

After filtration of the water samples, the virus-adsorbing filter holders were disconnected from the system, and sufficient pressure was applied to purge water remaining in the holders. Forty milliliters of 3% beef extract at pH 7.0 was then poured into each holder and allowed to remain in contact with the virus-adsorbing membrane for 30 min, after which the beef extract was forced through the membrane by air pressure, collected, and immediately stored at −70°C.

Solids collected on the prefilter were removed, taking care to transfer only a very fine top layer of the prefilter material. To these solids, pH 7.0-buffered 10% beef extract (1.35 g of Na_2HPO_4, 0.12 g of citric acid, 10 g of beef extract, and 100 ml of distilled water) was added at a ratio of 3 ml of beef extract to 1 g of solids. The mixture was stirred for 30 min and centrifuged at 2,500 × *g* for 15 min, and the supernatant was filtered

through a series of membrane filters (1.2, 0.65, and 0.45 μm) stacked in order of decreasing pore size in a Swinnex filter holder (Millipore Corp.).

From stations C-100, T-700, and T-600, a portion of the sample collected was processed with the Aquella virus concentrator seen in Fig. 3 (19). This particular model contained both honeycomb cartridge and flat disk filters. Cartridge prefilters included 5- and 1-μm Orlon filters and one 1-μm cotton filter and were loaded in that order. The virus-adsorbing cartridge filter was made of wound fiberglass with a 1-μm pore size. Filters had stainless-steel cores with the exception of the cotton filter which was constructed with a polypropylene core. The flat disk filter (267-mm diameter) was made of epoxy-fiberglass-asbestos (Cox type AA, M-780) with 5- and 1-μm pore sizes. Filters were stacked in the holder according to pore size, with the smaller pore size positioned on the bottom plate. All filters were autoclaved for 15 min at 121°C with the exception of the 1-μm cotton filter, which was sterilized by soaking in distilled water for 1 h at pH 1.0. To prevent adsorption of viruses to the cotton, we soaked this filter in a 1% Tween 80 solution for 1 h. After assembly the three prefilters were washed with chlorinated tap water to remove manufacturing sizings and lubricants, as well as excess Tween 80 solution. This was followed by a 20-liter wash with sterile distilled water containing sodium thiosulfate to neutralize residual chlorine.

Operation of the Aquella concentrator has been described elsewhere (19). In this study, the virus-adsorbing filters and the 5-μm Orlon prefilter were eluted. Because of the large volume of particulates collected from the C-100 samples, this prefilter was always eluted separately from the virus-adsorbing filters. In the case of the other sampling stations, these eluates were combined. The method of Wallis et al. (19) was used to elute the adsorbed virus. Filters and prefilters to be eluted were first washed with 0.14 M saline solution at pH 3.5, followed by elution of the viruses with 0.5 M (pH 11.5) glycine solution. The glycine was immediately adjusted to pH 3.5, and $AlCl_3$ was added to a concentration of 0.0005 M. The eluate was further concentrated on flat disk filters of 125- and 47-mm diameters. The 1- and 5-μm Cox type AA, 125-mm-diameter M-780 filters were positioned on the holder in order of decreasing pore size. The viruses were readsorbed, and the filters were then washed with 0.14 M saline solution at pH 3.5 and eluted with two 40-ml portions of 0.05 M (pH 11.5) glycine solu-

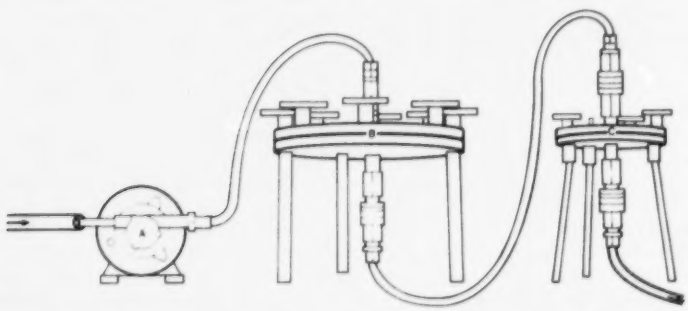

FIG. 2. *Disk adsorption virus elution system. (A) Water pump; (B) 293-mm prefilter holder; (C) 142-mm virus-adsorbing filter holder.*

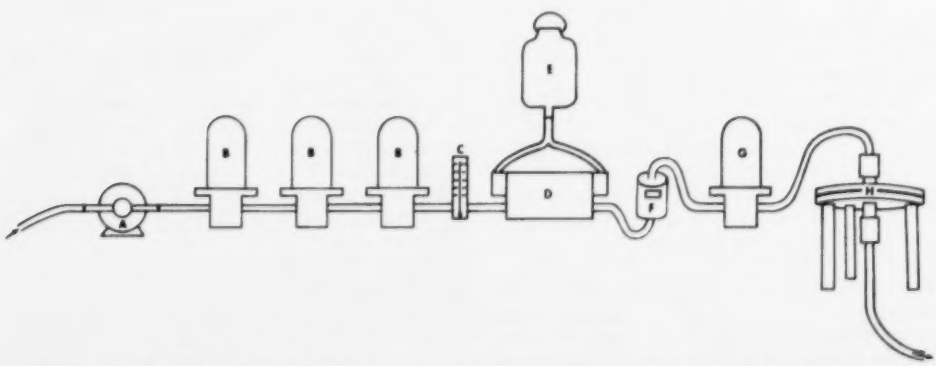

FIG. 3. *Flow diagram of Aquella virus concentrator. (A) Water pump; (B) cartridge prefilter holder; (C) rotometer; (D) fluid proportioner; (E) acid-salt reservoir; (F) water meter; (G) cartridge virus-adsorbing filter holder; (H) disk virus-adsorbing filter holder.*

tion. The eluate was immediately adjusted to pH 3.5, and $AlCl_3$ was added to a concentration of 0.0005 M. The reconcentrated eluate was passed through the 47-mm-diameter filters positioned in the holder with a Millipore MF, type HA filter on the bottom plate followed by the 1- and 5-μm Cox type AA, M-780 filters. The readsorbed viruses were washed as described above and eluted with two 5-ml portions of 0.05 M (pH 11.5) glycine solution. The eluate was immediately neutralized to pH 7.0, with pH 1.0 glycine and 0.5 ml of 3 M NaCl added to each 10 ml of the final concentrate.

Indicator bacteria analysis. Fecal coliform and fecal streptococcus determinations were conducted by the MF technique as described previously (7).

Storage and shipment of samples. Eluates from the processed river water and sewage samples were stored in sterile plastic bottles at −70°C, as were the solids after supplementation with beef extract. All samples were packed in Dry Ice and sent to the Cincinnati laboratory where they were immediately placed at −70°C.

RESULTS AND DISCUSSION

Sources of viruses. Sources of fecal pollution in the proximity of Fairbanks were assessed to determine the origin of enteric viruses in the T-700 to T-100 reaches of the Tanana River. An earlier report on this Tanana River study (7) called attention to negligible counts of fecal indicator bacteria above the confluence of local tributaries. Coupled with bacterial sampling of the T-900 station, we collected 380-liter samples for virus evaluation at two time periods. From the absence of detectable viruses at the T-900 station, we concluded that viral contribution from upstream sources could also be discounted. The earlier conclusion (7) that fecal contamination in the lower reach of the Tanana River was for the most part spewing forth from the Chena River (station C-100) could also be drawn from the substantial numbers of viruses recovered at this location (Table 1). This was further confirmed on determining that nearly 90% of waste treatment plant discharges empty into the Chena River in the Fairbanks area.

The most prevalent of the viruses typed were the polioviruses, as is true with virtually all domestic sewage effluents sampled since continuous use of oral polio vaccines. Table 1 shows that poliovirus comprised 83% of the viruses identified. The only other virus type consistently found was coxsackievirus B2, which totaled 14% of the typed viruses. Wastewaters entered the Chena River from the Fairbanks, College Utilities, and Fort Wainwright sewage treatment plants; from 27 to 65 infectious virus units were recovered in each liter of effluent discharged by these plants (Table 2). A measure of the viruses comprising these effluents (88% poliovirus and 11% coxsackievirus B2) revealed a high correlation between discharged viruses and those recovered from the Chena River. Table 2 also provides virus data on the other two domestic sewage plants with discharges into that section of the Tanana River studied. Finding coxsackievirus A16 and echoviruses 11 and 21 in the Chena River but not in discharges from the local sewage treatment plants indicates either a sporadic release of low levels of these viruses or a lessened sensitivity of the detection method.

TABLE 1. *Enteroviruses recovered from the Chena River sampling station (C100) by the disk adsorption virus elution procedure*

Day[a]	Virus content (PFU[b]/380 liters)	Viruses identified[c]					
		P	CB2	E25	E21	E11	CA16
1	208	13	9	0	0	0	1
2	176	25	3	0	0	1	0
8	262	41	4	0	1	0	0
9	106	24	1	0	0	0	0
10	246	23	4	1	0	0	0

[a] A total of 380 liters was collected at each sampling, with one-half of the volume concentrated by the disk adsorption virus elution procedure and the remaining half concentrated with the Aquella concentrator.

[b] PFU, Plaque-forming units.

[c] Of the plaques picked, all could be typed. Abbreviations: P, Poliovirus; CA, coxsackievirus A; CB, coxsackievirus B; E, echovirus.

Virus survival. Table 3 summarizes viral recovery from a 317-km reach of the Tanana River. The findings demonstrate the persistence of viruses far removed from sources of domestic pollution. Recognition that such distant sites may become contaminated emphasizes the inherent risk in assuming favorable conditions for self-purification of virus-laden water. There is general agreement that cold weather conditions prolong virus survival time. Thus persistence of these viruses, even after a mean flow time of 7.1 days, could have been anticipated from earlier laboratory studies (9). Moreover, virus inactivation profiles have suggested that their survival time is correlated in some way with the microbiological flora of natural water (9, 12). In a river environment of total ice cover and 0°C temperature, any influence of the normal river flora on the persistence of these viruses would be appreciably lowered.

Results of the hydrological data from each sampling station were presented in a prior report (7). Included in this data were the river discharge measurements from which the relative dilution factor at successive stations was derived. Factors of 1.71, 1.44, and 1.02 were determined for stations T-100, T-400, and T-600, respectively, based on a discharge dilution factor of 1.00 for

TABLE 2. *Recovery of enteroviruses from sewage treatment plant (STP) effluents discharged into tributaries of the Tanana River near Fairbanks, Alaska*

STP sampling location	No. of samples	Sample size (liters)	Virus content (PFU[a]/liter)	Viruses identified[b]			
				P	CB2	CB4	E25
Fairbanks	2	4	42	14	0	0	0
		3	27	7	0	0	0
College Utilities	2	3	53	8	3	0	1
		2	65	11	3	0	0
Ft. Wainwright	1	4	54	16	1	0	0
Eielson Air Force Base	1	2	63	10	4	0	0
North Pole	1	1.5	29	9	0	1	0

[a] PFU, Plaque-forming units.
[b] Of the plaques picked, all could be typed. Abbreviations: P, Poliovirus; CB, coxsackievirus B; E, echovirus.

TABLE 3. *Enteroviruses recovered from Tanana River sampling stations with the disk adsorption virus elution procedure*

Sampling station[a]	River profile from T700[b]		Day	Virus content (PFU[c]/380 liters)	Viruses identified[d]			
	Distance (km)	Mean flow (days)			P	CB2	E25	E21
T700	0	0	3	0	0	0	0	0
			4	2	1	0	0	0
			5	12	6	0	0	0
			8	10	4	1	0	0
			9	6	2	1	0	0
			12	8	4	0	0	0
T600	77	1.9	3	0	0	0	0	0
			4	2	0	0	0	1
			5	0	0	0	0	0
			8	2	1	0	0	0
			9	8	3	0	1	0
			12	22	8	2	0	1
T400	179	4.2	3	1	1	0	0	0
			4	0	0	0	0	0
			5	3	3	0	0	0
			9	2	2	0	0	0
			12	3	3	0	0	0
T100	317	7.1	3	0	0	0	0	0
			4	0	0	0	0	0
			8	3	3	0	0	0
			9	2	2	0	0	0

[a] At each sampling, 380 liters was collected. The total volume collected from stations T400 and T100 was concentrated by the disk adsorption virus elution procedure. The samples collected from stations T700 and T600 were divided; one-half of the volume was concentrated by the disk adsorption virus elution procedure, and the remaining half was concentrated by using the Aquella concentrator.
[b] Hydrological data are as described by Davenport et al. (7).
[c] PFU, Plaque-forming units.
[d] Of the plaques picked, all could be typed. Abbreviations: P, Poliovirus; CB, coxsackievirus B; E, echovirus.

the T-700 station. With an adjustment of the calculated mean values in Table 3 for dilution, 34% of the initial virus population survived passage from station T-700 through T-100.

Only polioviruses were observed at the T-100 and T-400 stations. Assessment of virus types in the Chena River, from which most of the fecal pollution emanated, gave a relatively high poliovirus density (Table 1). With 83% of the identified viruses being of this type and the relatively low total virus recoveries at the T-700 station, the absence of other virus types at downstream sampling stations seems consistent with the sensitivity of the detection procedure. Although virus recoveries at stations T-700 and T-600 were subject to large variations on different days, such distributions are not uncommon in virus patterns observed in other polluted waters

(2, 14).

Enteric viruses recovered with the disk adsorption virus elution procedure were, for the most part, from solids retained on the prefilters. The capacity of viruses to adsorb to solids is well known (15). What is unanswered is whether these viruses were already bound to waterborne solids or became adsorbed to solids that accumulated on the prefilters.

Table 4 shows the results of virus recovery from those stations in which a portion of the sample was processed with the Aquella virus concentrator. Overall viral recoveries were appreciably lower than those obtained with the disk adsorption virus elution procedure. However, the two exceptions (station T-700 on days 3 and 4) point to the variables inherent in processing natural waters. Since these studies, there have been substantial improvements to the Aquella virus concentrator. Thus, caution should be exercised in interpreting the effectiveness (for field application) of the two methods utilized in this study.

Indicators of enteric viruses. Table 5 compares the virus and fecal coliform populations in treated wastewater discharges. Among treatment plants in this study, ratios of enteric virus-to-fecal coliforms varied from 1:34,900 to 1:3,700,000. Clearly, the coliform population gave no quantitative measure of virus contamination present in these discharges—a conclusion previously drawn by Berg and Metcalf (3), who dismissed as insensitive the use of a constant ratio between fecal indicator bacteria and enteric viruses in sewage and surface waters. Differences in excretion patterns alone dispel any likelihood that the numbers of fecal indicator bacteria would have provided a reliable measure of the numbers of viruses present. It is important to recognize that fecal indicator bacteria are excreted by all sections of the population in contrast to viruses which are excreted only by infected individuals.

Monitoring the Chena River (C-100) offered a direct means for studying the course of most of the fecal pollution channeled into that section of the Tanana River studied. By weighing the discharge levels of the plants emptying into the Chena River, it was calculated that the virus-to-fecal coliform ratio was about 1:2,616,000, or more than five times the figure reported for the C-100 station (Table 6). Thus, fecal coliform die-off in the Chena River occurred at an appreciably faster rate than that of enteric viruses. This greater die-off rate has not always been recognized as a constant pattern in all receiving waters. As submitted by Geldreich and Clarke (8), fecal coliforms appear to survive relatively longer than enteric viruses in some waters. Although they indicated no constant relationship exists between viruses and fecal coliforms in natural waters, others have reported that viruses generally have the greater survival capacity (3, 12).

In the Tanana River the virus-to-fecal coliform ratios ranged from 1:314,000 to 1:738,000 (Table 6). In contrast to these results, studies of the Missouri and Mississippi Rivers revealed ratios that ranged from 1:14,000 to 1:7,600,000

TABLE 4. *Enteroviruses recovered from river sampling stations with the Aquella concentrator*

Sampling station[a]	Day	Virus content (PFU[b]/380 liters)	Virus identified[c]	
			No.	Type
C100	1	20	10	Poliovirus
	2	—[d]	—	
	8	2	1	Poliovirus
	9	12	6	Poliovirus
	10	4	2	Poliovirus
T700	3	2	1	Poliovirus
	4	2	1	Poliovirus
	5	4	2	Poliovirus
	8	0	0	
	9	0	0	
	12	0	0	
T600	3	0	0	
	4	0	0	
	5	0	0	
	8	0	0	
	9	0	0	
	12	0	0	

[a] A 380-liter sample was collected from stations C100, T700 and T600 at each sampling. One-half of the volume collected was concentrated with the Aquella concentrator and the remaining one-half was concentrated by the disk adsorption virus elution procedure.

[b] PFU, Plaque-forming units.

[c] Of the plaques picked, all could be typed.

[d] —, Not tested.

TABLE 5. *Ratio of enteroviruses to fecal coliforms (FC) in sewage treatment plant (STP) effluents*

STP sampling location	No. of samples	Virus content (PFU[a]/100 ml)	FC (count/100 ml)	Ratio (virus:FC)
Fairbanks	2	4.2	7.8×10^6	1:1,850,000
		2.7	1.0×10^7	1:3,700,000
College Utilities	2	5.3	4.8×10^6	1:905,000
		6.5	2.5×10^6	1:385,000
Ft. Wainwright	1	5.4	1.9×10^7	1:3,510,000
Eielson Air Force Base	1	6.3	2.2×10^5	1:34,900
North Pole	1	2.9	3.5×10^5	1:121,000

[a] PFU, Plaque-forming units.

TABLE 6. *Comparison of the arithmetic mean of enterovirus, fecal coliform (FC), and fecal streptococcus (FS) counts from river sampling stations*

Sampling[a] station	Mean counts/380 liters			Ratio (virus:FC:FS)
	Enteroviruses[b]	FC[c]	FS[c]	
C100	235	113×10^6	931×10^4	1:481,000:39,600
T700	6.33	467×10^4	473×10^3	1:738,000:74,200
T600	5.67	188×10^4	240×10^3	1:332,000:42,300
T400	1.80	820×10^3	135×10^3	1:456,000:75,000
T100	1.25	393×10^3	732×10^2	1:314,000:58,600

[a] Mean of samples collected on days 1 and 8 for station C100; days 3, 4, 5, 8, 9, and 12 for stations T600 and T700; days 3, 4, 5, 9, and 12 for station T400; and days 3, 4, 8, and 9 for station T100.

[b] Mean calculated from Tables 1 and 3.

[c] Data are as described by Davenport et al. (7).

(3). The nearly 18-fold difference in the virus-to-fecal coliform ratios in the two studies may be reconciled solely by the fact that in the section of the Tanana River studied, all fecal pollution enters from upstream sources, whereas in the Missouri and Mississippi Rivers sewage from point and nonpoint sources enters over the entire course of their flows.

The disparity in the die-off ratios of viruses and fecal coliforms in the Tanana River may have reflected the high virus recovery at station T-600 on day 12. The enteric viruses nevertheless showed a greater capacity to survive in this water environment, although not to the same degree as seen in the Chena River. This difference in the relative die-off rates of the Chena and Tanana Rivers may rest with earlier observations that a grossly sewage-polluted water will generally prolong virus survival (9).

Fecal coliforms showed a lower survival rate than fecal streptococci (Table 6). At the T-700 station the fecal coliform-to-fecal streptococcus ratio was 9.95:1; at the T-100 station the ratio had decreased to 5.36:1. Kenner (10) reported that fecal coliform-to-fecal streptococcus ratios of 4 or greater were indicative of human pollution. Our results corroborated this finding. By taking into account the relative dilution factor at station T-100, survival rate of fecal streptococci (26%) was more closely related to that of the enteric virus (34%) than was the survival rate of fecal coliforms (14%) in this 317-km reach. Others have likewise reported that fecal streptococci were generally more persistent than coliforms (5, 7, 10). Because their persistence parallels viral survival more closely than fecal coliforms, Cohen and Shuval (5) suggest that fecal streptococci may be better indicators of virus in less polluted waters.

Results in this study leave no doubt that viral risks posed by the Tanana River were indicated by the numbers of fecal coliform and fecal streptococci also present. However, Berg (2) has pointed out that there are conditions in which viruses have persisted in the apparent absence of fecal indicator bacteria.

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